1935

BULLETIN 303 JUNE, 1935

Investigations on Downy Mildew of Tobacco

G. M. ARMSTRONG AND C. B. SUMNER

IMPERIAL MYCOLOGICAL INSTITUTE
9 OCT 1935

SOUTH CAROLINA
AGRICULTURAL EXPERIMENT STATION
OF
CLEMSON AGRICULTURAL COLLEGE
H. W. BARRE, Director
Clemson, South Carolina

EXPERIMENT STATION STAFF

BOARD OF CONTROL

HON. J. E. WANNAMAKER, St. Matthews, S. C., *Ex Officio**
HON. A. F. LEVER, Columbia, S. C.
HON. PAUL SANDERS, Ritter S. C.
HON. CHRISTIE BENET, Columbia, S. C.
HON. SAM H. SHERARD, Ninety Six, S. C.
HON. T. B. YOUNG, Florence, S. C.

E. W. SIKES, *President*
R. A. MCGINTY, *Acting Director*

H. W. BARRE, *Director*†
G. H. AULL, *Asst. Director*†

AGRICULTURAL ECONOMICS

G. H. AULL, *Agri. Economist*†
B. O. WILLIAMS,
Associate Rural Sociologist
Acting Head of Department
MARVIN GUIN, *Asst. Agri. Economist*
H. A. WHITE, *Asst. Agri. Economist*
J. L. FULMER, *Asst. Agri. Economist*
ERNEST RILEY, *Asst. Agri. Economist*

AGRONOMY

H. P. COOPER, *Agronomist*
W. R. PADEN, *Associate Agronomist*
W. B. ROGERS, *Assistant Agronomist*†
B. E. G. PRITCHARD,
Assistant in Agronomy†
R. W. LIPSCOMB, *Asst. in Agronomy*
T. C. PEELE, *Associate Soil Scientist*

ANIMAL HUSBANDRY

L. V. STARKEY, *Animal Husbandman*
E. G. GODBEY, *Assoc. Animal Husb.*

BOTANY AND BACTERIOLOGY

G. M. ARMSTRONG,
Botanist and Plant Pathologist
W. B. AULL, *Asso. Bacteriologist*
C. H. ARNDT, *Assoc. Bot. and Plant Path,*
C. B. SUMNER,
Assistant in Plant Pathology
C. C. BENNETT, *Laboratory Assistant*

CHEMISTRY

J. H. MITCHELL, *Chemist*
D. B. RODERICK, *Assistant Chemist*
W. B. KELLER, *Assistant in Chemistry*

CROP PEST COMMISSION

G. M. ARMSTRONG, *State Pathologist*
FRANKLIN SHERMAN, *State Entomologist*
J. A. BERLY, *Asst. State Entomologist*
M. B. STEVENSON, *Asst. State Pathologist*

DAIRY

J. P. LAMASTER, *Dairyman*
E. C. ELTING, *Associate Dairyman*
W. H. PADGETT, *Supt. of A. R, Testing*

ENTOMOLOGY

FRANKLIN SHERMAN, *Entomologist*
O. L. CARTWRIGHT,
Associate Entomologist
J. G. WATTS, *Asst. Entomologist*
J. N. TODD, *Assistant in Entomolgy*

HOME ECONOMICS

Rock Hill, S. C.
MARY E. FRASER, *Home Economist*
ADA M. MOSER,
Research Spec. in Home Econ.

HORTICULTURE

A. M. MUSSER, *Horticulturist*

FARM

C. S. PATRICK, *Head Farms Department*

POULTRY

C. L. MORGAN, *Poultryman*
D. F. SOWELL,
Assistant in Poultry Husbandry

PUBLICATIONS

A. B. BRYAN, *Agricultural Editor*

VETERINARY

R. O. FEELEY, *Veterinarian*

COAST STATION

Summerville, S. C.
E. D. KYZER, *Superintendent*
T. M. CLYBURN, *Agent*
(U. S. Bureau Animal Industry)

PEE DEE STATION

Florence, S. C.
E. E. HALL, *Superintendent*
W. B. ALBERT, *Asso. Plant Physiologist*
FRANK HARRELL, *Research Assistant*
F. F. BONDY,, *Asst. Entomologist*
(U. S. Bureau Entomology)

SANDHILL STATION

R. 5, Columbia, S. C.
J. A. RILEY, *Superintendent*
L. E. SCOTT, *Asst. Horticulturist*
R. W. WALLACE, *Assistant Agronomist*
N. MCKAIG, JR.,
Associate Soil Technologist
(U. S. Bureau Chemistry and Soils)
E. W. FAIRES, *Agent*
(U. S. Bureau Dairying)
E. C. ROLLER, *Assistant Chemist*
(U. S. Bureau Chemistry and Soils)

TRUCK STATION

P. O. Box 337, Charleston, S. C.
W. D. MOORE,
Asso. Plant Pathologist, in Charge
(Bureau of Plant Industry)
J. M. JENKINS, *Assistant Horticulturist*
FRANCIS E. JOHNSTONE, JR.,
Assistant Pathologist
W. J. REID, JR., *Asst. Entomologist*
(Bureau of Entomology)

*Deceased, March 5, 1935
†On leave.

TABLE OF CONTENTS

Investigations on Dowy Mildew of Tobacco*

G. M. ARMSTRONG AND C. B. SUMNER

INTRODUCTION

Downy mildew, or blue mold (*Peronospora tabacina* Adam), of tobacco probably made its first appearance in South Carolina in the spring of 1931, since it was known to have been in the neighboring states of Georgia and North Carolina at that time. In the spring of 1932, however, this disease appeared with devastating effects. As a result of the earliness of the attack and the rapid spread of the disease during 1932, a large proportion of the seedlings were killed and plants were very scarce at transplanting time. The ultimate result of this outbreak was a reduction in yield of approximately 40 per cent. The disease has made its reappearance each year since 1932, though the losses in subsequent years have not been great. It has appeared in the tobacco plant beds at the Pee Dee Experiment Station at Florence, South Carolina, at approximately the same date each year but since 1932 its spread has been delayed to such an extent that most plants have grown sufficiently large to escape serious damage.

It is obvious that this disease has become permanently established in South Carolina and is a constant threat to the tobacco growing industry. While the actual damage done since 1932 has not been great, the fungus is present and with favorable environmental conditions may produce another destructive outbreak.

The present investigation was begun as a result of the disastrous outbreak of downy mildew in 1932 and has been continued to the present time. This publication gives the results of this study, together with a brief history and description of the disease.

HISTORY OF THE DISEASE

The downy mildew of tobacco is supposed to have appeared originally in Australia, although Farlow was the first to record a downy mildew on Nicotiana which was collected in California in 1885 (9). The first record of the disease in Australia was not made until 1890 (17), but reports of the older growers indicate that it was present at least 30 to 40 years earlier. Since 1890, however, it has developed into a major problem of the tobacco growers in Australia. Although reported in the United States in 1885, the disease did not attack commercial tobacco until the spring of 1921, appearing then in the shade-grown tobacco area of Florida and Georgia (13). It was not reported again until the spring of

*Acknowledgment is due Dr. W. B. Albert for aid in the investigations at Florence in 1933. Mr. J. C. Burton assisted in the attempts to germinate oospores.

1931. Since its reappearance, it has been found in Louisiana, Florida, the flue-cured tobacco area of the Southeast, Tennessee, Maryland, and southeastern Pennsylvania.

SYMPTOMS OF THE DISEASE

The symptoms of the disease are well known to practically every tobacco grower in the regions where it occurs. Occasionally it is confused with other troubles, but usually the characteristics are so outstanding that it can be readily identified. As a rule the first symptom is drooping of the tips of the infected leaves followed by yellowing, either in spots or over the entire leaf. Almost immediately, under favorable weather conditions, the fruiting bodies of the fungus produce a dense downy coating on the under surface of the leaves, thus distinguishing it from other tobacco diseases. A warm dry day following the appearance of the downy growth causes the infected area to die and become dark in color, and the plants soon present the appearance of having been scalded with hot water. The fungus is unable to produce fruiting bodies if warm and dry weather prevails for several days after infection has occurred. During such periods yellow flecks or spots appear on the leaves, in which a coenocytic mycelium has been found in abundance. Microscopic examination of these spots will occasionally show the presence of fruiting bodies but these do not occur in groups dense enough to be visible otherwise.

Plants that are quite small are usually killed when attacked but somewhat larger ones frequently survive after infection has occurred. All of the leaves except those around the bud may drop off but in one to three weeks these plants may have recovered to such an extent that few evidences of the disease are noticeable.

In the experimental work conducted in the temperature-humidity cases, occasionally a soft rot has been encountered. This soft rot is caused by a bacterium and occurs only with the downy mildew lesions or in mechanically injured leaves. It has been found in the seed beds as well but is of minor importance.

TEMPERATURE-HUMIDITY CASES

Two cases, in which the temperature and the humidity can be controlled, were constructed in the spring of 1933, thus enabling a year-round study of the disease. This apparatus was necessary, since the casual organism is an obligate parasite requiring cool, moist conditions for its cycle of development. With the advent of hot weather, the conidial stage of the fungus disappears.

The two cases, one of which is shown in Figure 1, are modifications of those described by Stoughton (16), with walls of double glass and outside dimensions 34 inches high, 29 inches deep, and 47 inches wide. The front of each box is composed of two double glass doors, the top is essentially a glass bottomed pan, and the bottom is of metal with a small drain in the center.

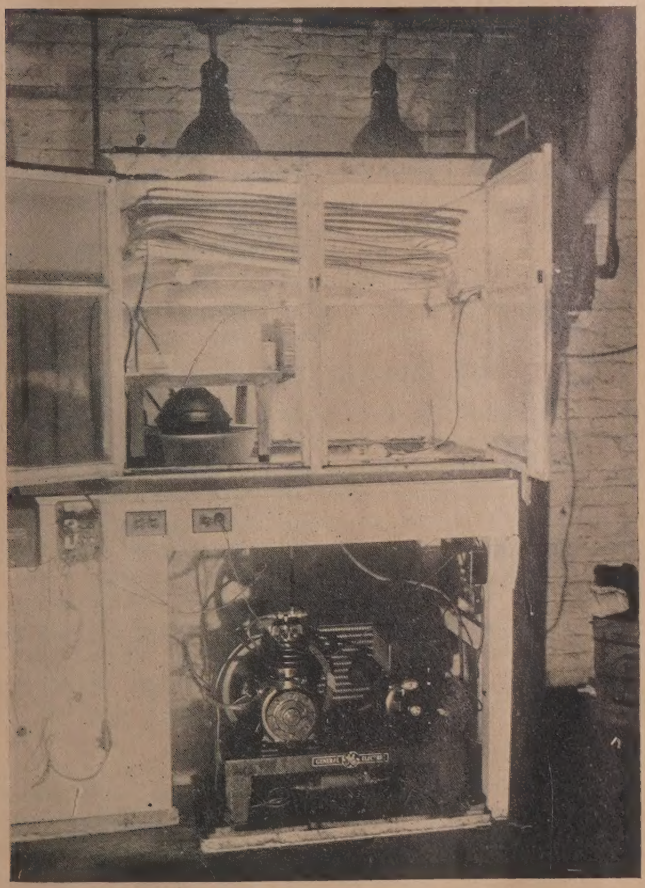

Figure 1.—A temperature-humidity case.

Low temperatures are obtained by pumping cold water from a storage tank outside the case through a copper pipe coiled around the top portion of the side walls of the case. A thermostat inside the case, through a relay, controls the circulation by turning the pump motor off or on as needed. A small refrigeration unit cools the water of the storage tank to 7° to 10°C., and the lowest temperature obtainable in the case is 13°C. due to the temperature of the room in which it is kept. A temperature higher than that of the room is obtained by connecting the thermostat to a heating unit in the case instead of the pump.

In one case, moisture is introduced through a small Gilbert humidifier, controlled through a relay by a Friez humidistat. This outfit is accurate at lower ranges but becomes erratic in behavior when set to produce relative humidities above 90 per cent.

In the other case, a fine mist of water is introduced at one side through a "mistor head" nozzle commonly used by retail vegetable dealers to maintain the freshness of their products. Only the inoculated plants placed nearest the source of spray in about half the area of this case will develop the disease.

Illumination is provided by using two standard 500-watt bulbs suspended over the glass top of the case. Heat is absorbed by separating bulbs and case by a stream of water flowing over the top of the case and through a drain at one end.

THE CAUSAL ORGANISM

The fungus *Peronospora hyoscyami* de Bary has frequently been associated with the downy mildew of tobacco though it is now known that the Peronospora on tobacco will not infect *Hyoscyamus niger,* from which de Bary obtained the organism which he described. Spegazzini (14) named a fungus attacking tobacco in Argentina *Peronospora nicotianae.* Angell and Hill (2) were uncertain whether the fungus in Australia should be called *P. nicotianae* Speg. or described as a new species. Wolf et al. (18) have used the name *P. nicotianae* Speg. for the fungus. Adam (1) has recently shown that the Australian downy mildew on tobacco is a new species, *P. tabacina,* and Clayton and Stevenson (6) have concluded that the fungus occurring in the United States is also this species.

HOST PLANTS

Wolf et al. (18) give a list of the plants on which the tobacco downy mildew fungus has been found. Armstrong and Albert (3) reported the fungus on tomato, *Lycopersicum esculentum,* pepper, *Capsicum annuum,* and eggplant, *Solanum melongena.* They were successful in producing the disease in pepper by cross-inoculations with conidia obtained from tobacco. In 1932, severe losses from this disease occurred in pepper plant beds in the vicinity of Florence though losses since that time have been insignificant. Artificial inoculations of dozens of tomato, pepper, and eggplant have been made in the temperature-humidity cases at irregular intervals during the past 18 months. All conidia for the inoculations were obtained from tobacco. Lesions appeared on the leaves in a few days after the inoculations but in no instance, except on one tomato leaf, have examinations with the naked eye or a hand lens revealed conidia. The eggplant, pepper, and tomato plants were inoculated with some of the same inoculum and kept in the same moist chambers with tobacco which developed the disease abundantly, yet with a single exception, no conidia were found on the first mentioned hosts. In May 1934, seedlings of eggplant, pepper, and tomato

were taken to beds containing diseased tobacco plants, thoroughly inoculated, and then left in the shade at the edge of a tobacco bed. At the end of five days conidia were produced on tomato and eggplant but not on pepper, though conidia did appear on pepper on the seventh day. In April 1934, 19 of 20 tobacco plants were infected by inoculating with conidia obtained from peppers.

Artificial inoculations of black nightshade, *Solanum nigrum*, and horsenettle, *S. carolinense*, failed to produce the disease.

MORPHOLOGY

The mycelium of this fungus is coenocytic in common with other Phycomycetes. It is extremely irregular in diameter, intercellular in habit, and produces many haustoria in the leaf tissues. Instead of being coiled as described by Angell and Hill (2) the haustoria are generally branched under South Carolina conditions. Conidia and conidiophores are of the typical Peronospora type. Oospores were produced fairly abundantly in most of the diseased leaf material examined. Leaves of approximately equal size from young plants, collected the morning conidia appeared, were kept in moist petri dishes both at room temperature (24° to 25°C.) and at 16° to 20°C. Twice daily thereafter, some of the leaves were cleared in lactic acid and examined microscopically. On the fourth day, in both sets, globular, hyaline bodies were plainly evident. On the sixth day these bodies had the appearance of mature oospores.

PHYSIOLOGY

GERMINATION OF CONIDIA

Four separate series of conidial germinations have been made. Conidia were placed in drops of tap water on slides and kept in moist petri dishes at 12 temperatures ranging from 1° to 35°C. One series was terminated at the end of 22 hours and the others at the end of 48 hours. The drops of water were brought to the desired temperatures before the conidia were added. The greatest germination at the temperatures of 1° to 3°C. and 27° to 29°C. were 15 and two per cent respectively at the end of 48 hours. No germination was observed at higher temperatures. The highest percentages of germination were noted at temperatures of 15° to 23°C. inclusive, the differences in germination within this range being insignificant.

In the course of the routine inoculations of plants in the temperature-humidity chambers, spore germinations were frequently made and fresh conidia generally showed very high percentages of germination in 24 hours, with little or no change during an additional 24 hours (temperature 16° to 20°C.). In at least five instances, however, rather pronounced delayed germination occurred between the 24-hour and 48-hour observations. On two occasions

with somewhat dark and relatively old conidia there was no germination in 24 hours but 15 per cent and 20 per cent at 48 hours. After fresh spores were placed in water an accidental rise in temperature from 16° to 30°C. occurred in 2½ hours and was maintained for 15½ hours. Less than one per cent germination was noted. With the temperature at 16°C. during the next 24 hours the germination increased to 90 per cent. The blue color of conidia so frequently observed in plant beds is decidedly uncommon in the temperature-humidity chambers, having been observed in only a few instances, and the percentage of germination of such conidia was low. In one instance of delayed germination, 10 per cent and 60 per cent of the conidia from blue areas had germinated in 24 and 48 hours respectively.

On two different days, one cloudy and relatively cool, the other bright and relatively warm, infected leaves from plant beds were brought into the laboratory at hourly intervals and germination tests were made with the conidia from these leaves. The results were decidedly irregular but showed that viable conidia were present at all hours of the day, from 8 A. M. to 5 P. M., the 5 o'clock collection on the cloudy day giving 80 per cent germination.

To test the viability of conidia found on various types of lesions, leaves were brought into the laboratory at noon on a cloudy day and germination tests in duplicate were made with conidia from the following types of lesions: apparently fresh conidia; conidia from bluish spots; conida from dead dry areas; and conidia from small leaves showing soft rot. Again the results were irregular but appreciable germination was obtained in each case.

Conidia germinated on dry glass slides at relative humidities of 98.2 per cent and above but not at lower relative humidities. Different relative humidities were obtained by sulfuric acid—water mixtures as given by Stevens (15). The dry slides with conidia were placed above these mixtures in moist chambers sealed with vaseline.

ATTEMPTS TO GERMINATE OOSPORES

It appeared that the rapid spread of the disease in 1932 must have been accomplished through the dissemination of the conidial stage of the downy mildew fungus. Wolf et al. (18) and Dixon et al. (8) have presented evidence to show that the earliest infections may occur from the germination of the heavy-walled, overwintering oospores and the secondary spread of the fungus follows by means of the conidia. If the oospores could be germinated, further evidence as to the source of early infections would be indicated. The report of Bressman and Nichols (4) that two-year-old dried herbarium material of the hop downy mildew yielded oospores which would germinate in water led to similar attempts with tobacco downy mildew oospores. Using the methods suggested by them (4), numerous attempts to germinate oospores have been

made. One-year-old and two-year-old herbarium material, unfrozen and frozen for varying periods; material collected in the spring, stored at room temperature until late August, and then held in a refrigerator at about 7°C. for four months, and some of it later frozen; material collected from the fall experiments, and kept in cheese cloth bags out of doors in plant beds until spring; and fresh material have all been variously treated and used in the germination trials. Microscopic observations in all instances and plant inoculations in many instances were used to ascertain if germination occurred. Oospores, either in drops of water on slides or in petri dishes with moist filter paper, have been kept for varying periods at room temperature, in the temperature-humidity cases at 16° to 20°C., and in the refrigerator at about 7°C. Since negative results were obtained with all these tests, substances used to break the rest period of hard seed, buds, corms, and tubers, were then tried (12). These substances have been used in several concentrations for different periods of time but without success in producing oospore germination. In general, the oospores were given a vapor treatment if the chemical was volatile, otherwise they were placed in the solution. Some of the substances used for treatment were: ethylene chlorohydrin, ether, potassium thiocyanate, sulfuric acid, carbon disulfide, furfural, potassium nitrate, methyl mercaptan, thioacetamide, thiosemicarbazide, sodium amytal, methyl disulfide, and silt suspension from a plant bed.

HUMIDITY IN RELATION TO INFECTION AND DEVELOPMENT OF THE DISEASE

The downy mildew of tobacco occurs in the spring when the weather is cool and the plants are wet with dew much of the night and often late into the day. These moist conditions have been commonly observed to be very favorable to the production of the disease. It has been stated, also, that the conidia will not form except in the present of a film of water on the leaf surfaces. The following experiments were undertaken to determine the effect of humidity on infection and development of the disease.

Six inoculated plants, which were kept in a moist chamber for 48 hours to obtain infection, were freed of moisture and placed in a temperature-humidity case at 16° ± 1°C. A hygrothermograph showed variations in the relative humidity from 78 to 89 per cent while the plants were in this case. Conidia were produced under these conditions with no visible moisture on the leaves. To determine if both infection and sporulation would occur under the above conditions, other plants in individual pots were placed in the case for 30 minutes and then inoculated by lightly drawing diseased leaves over the healthy ones. At the end of nine days, five leaves from five plants showed dry papery areas characteristic of the disease. Conidiophores and conidia were not sufficiently abundant to

be clearly seen with the naked eye but a microscopic examination showed fruiting conidiophores protruding from stomata. Several other plants have produced conidia at various times in this case which maintains a relatively high humidity, being, however, less than the dew-point.

TEMPERATURE IN RELATION TO INFECTION AND DEVELOPMENT OF THE DISEASE

Before the construction of the temperature-humidity cases, three tests were performed to determine the temperature range for infection. A long, well insulated, copper-lined chamber with an ice-salt mixture at one end and heated water at the other was available. The chamber was divided into 12 compartments, each with a thermometer projecting through the lid. The temperature range was from 1° to 37°C. The temperature in each compartment was generally held within a range of 3° to 4°C., or less. Small succulent potted plants were inoculated, kept in the chambers for 48 hours, and then placed outside under conditions favorable for the development of the disease. In two of the three tests, plants from the chamber at 5° to 8°C. developed the disease, but no infection occurred at lower temperatures. The highest temperature at which infection occurred was 22° to 24.5°C. in one test and 27° to 28.5°C. in the other two tests.

Several tests also were made at various temperatures in the temperature-humidity cases, where the inoculated plants were kept at a constant temperature throughout an experiment.

Two tests using 10 plants each failed to show any symptoms of disease when kept for 12 days at a constant temperature of 31.5° to 32°C. Infection occurred in 12 plants kept at 30°C. but no conidia were found.

Abundant infection and production of conidia occurred on the two smallest of 10 small succulent plants kept at 25° to 26°C. and some fruiting occurred on four leaves of the larger plants. A repetition of this experiment, using somewhat older and less succulent plants gave only two infected leaves and the production of conidia could be determined only with the aid of a microscope.

A test with 30 plants at 21.5°C. gave abundant sporulation in eight days. These results are not in agreement with those of Clayton and Gaines (5) which indicate that sporulation would not occur above 70°F. (21°C.).

PRODUCTION OF CONIDIA AT INTERVALS ON OVERWINTERING PLANTS

A periodic outbreak of sporulation of the fungus was noticed during the winter of 1933-34 on 20 plants in a bed used in the fall experiments. These were the only plants remaining in an inoculated and unheated bed, where all other plants had been killed by the

disease. A glass sash partly covered this bed throughout the winter, allowing the plants to survive. At the time of the first freezing weather on November 9, no symptoms of the disease could be found. Sporulation of the fungus was first noticed November 25 and each day thereafter until December 8. Sporulation was noted on four other occasions during the winter, viz., January 6, 15, February 13, 23, the duration of the fruiting period of the fungus varying from one to three days each time.

In the fall of 1934, four beds, each containing 15 plants known to have had the disease, were selected in order to check the observations of periodic sporulation during the previous winter. Two of these beds were partly covered with glass and the other two by three thicknesses of cheesecloth on frames. After freezing weather on November 10, the plants were examined every few days. Sporulation of the fungus occurred under both the glass and cheesecloth covers, the first being noted December 5 on a few leaves under glass. The following day these spots had dried and no other sporulation could be found. Fresh conidia were found subsequently on December 17 to 19 inclusive; January 3 to 6 inclusive; January 18 and 19; February 3 to 5 inclusive; February 21; and March 3 and 4.

On the other hand, in the last several months, diseased plants have been allowed to recover in the cases and left for at least a month without showing a recurrence of symptoms. Other diseased plants have been removed and then returned to the cases without inoculation and in no instance have the symptoms of the disease reappeared. In several of these plants, however, a large coenocytic mycelium has been found in leaves and stems that were healthy in appearance. Apparently, the conditions necessary for periodic sporulation are not reproduced in the temperature-humidity cases. There is no definite proof that the sporadic sporulation on plants in the beds which occurred during two winter seasons was not the result of reinfections, but the intervals between the appearance of conidia indicate that they were most probably produced from the fungus persisting within the plant. This explanation agrees with the statement of Darnell—Smith (7) that a plant once infected with the downy mildew fungus is always a potential source of conidia.

REINFECTION OF PLANTS WHICH HAVE RECOVERED FROM THE DISEASE

Certain field observations have indicated that plants which have recovered from an attack of the disease are not subject to further infection and this opinion seems to be generally held by the tobacco growers. Other observations, and experimental evidence presented in this paper show, however, that this is not always true. In the spring of 1933 the reinoculation of plants which had recovered from the disease in beds at Florence failed to produce symptoms of the disease even though it was present in nearby beds and weather conditions were apparently favorable for its development. Also, in the

spring of 1933, the senior writer and Mr. W. M. Lunn of the United States Department of Agriculture transported several recovered plants, three to four inches high, from the beds at Florence, South Carolina, to the beds at Oxford, North Carolina. These plans had recovered from the disease and were carefully lifted with sufficient earth on the roots to reduce the shock of transplanting to a minimum. The disease was just appearing at Oxford and ultimately swept through the beds, infecting practically every plant except those from Florence.

Several large plants which had recovered from the disease were potted, placed in the temperature-humidity cases, and inoculated with conidia of the fungus. Although allowed to remain in these cases for three to four weeks under conditions favorable to the development of the fungus, no symptoms of the disease appeared. Recently, however, several infections have been obtained on similar plants by enclosing the large inoculated leaves with cellophane bags.

In early June 1934, a second epidemic of the disease appeared in the beds at Florence several weeks after the first epidemic had apparently disappeared. Also, on June 7, when the writers visited Florence, they found a field infection centered in four rows of 36 plants each, involving 17 plants, with scattered infections nearby. The disease appeared to have been present throughout the beds from which these plants were taken. These observations, however, are not sufficient in themselves to indicate whether reinfection occurred, or whether coniodophores and conidia may have developed from an internal mycelium. They are included partly to record a definite though limited outbreak of the disease in the field.

An experiment was performed to determine if recovered plants could be successfully reinoculated under artificial conditions. Twenty-four five-inch pots, each containing seven to nine plants, were used. These pots were divided into four series of six pots each, designated as series A, B, C, and D, and treated as described in Table 1.

It will be noted from Table 1 that the plants in series A were successfully inoculated three times, series B two times, and Series C one time. Some plants were killed as a result of each inoculation but a sufficient number survived to give convincing evidence that all diseased plants, after having recovered, are not immune to further infection. Subsequent to this experiment, in several instances, diseased plants were tagged and left to recover either in the cases or in the greenhouse. Upon being reinoculated, most of these plants showed the disease in about a week, though some of them showed no symptoms of the disease. In the routine inoculations, it has been noted that a succulent plant seems most susceptible to the disease and a young hardened plant is much less susceptible than a somewhat older succulent plant. The conditions within the plant,

Table 1.—Infection of Plants Followed by Reinfection After Recovery From the Disease

Date	Series A	Series B	Series C	Series D
8-20-34	Inoculated, placed in battery jar moist chambers, and in temperature-humidity cases.	Series B, C, and D treated the same as series A, except uninoculated.		
8-29-34	All plants showing definite symptoms of disease; removed from cases and moist chambers to a basement room.	Removed series B, C, and D from cases and moist chambers; no signs of disease. Placed in basement room to recover from shock of removal from saturated atmosphere.		
8-30-34	Removed to greenhouse for recovery.	Removed series B, C, and D to separate greenhouse from that of series A.		
9-9-34	Returned to cases; inoculated series A second time.	Inoculated series B for first time and treated the same as series A.	Returned to cases; uninoculated as on 8-20-34.	
9-16-34	All plants showing fruiting of the fungus.	All plants showing fruiting of the fungus.	All plants healthy.	
9-16-34 9-17-34	Series A and B treated the same as series A on August 29 and 30.		Series C and D first placed in basement room and then in separate greenhouse from A and B.	
9-28-34	Returned to cases; inoculated series A third time.	Returned to cases; inoculated B second time and treated as before.	Returned series C to cases; inoculated first time.	Returned to cases uninoculated.
10-6-34	All plants in series A, B, and C showing disease symptoms.			Healthy

Experiment discontinued because large plants could no longer be placed in moist chambers.

therefore, in addition to the conditions of the environment alone are important factors in determining infection and the development of the disease.

METHODS OF CONTROL

Three general methods have been tried for the direct control of downy mildew, viz., constant high temperatures; forced air ventilation; and spray mixtures. These will be discussed in detail.

HIGH TEMPERATURES

Five separate experiments were performed on the effect of continuously high temperatures upon the control of the disease, three at Clemson and two at the Pee Dee station at Florence. The first experiment of this type was made at Florence in April 1933. An electric hot bed heating cable was used as a source of heat and heavy cloth as a bed cover and heat retainer. The disease appeared sooner and caused greater damage in this bed than in the adjacent unheated beds. The record of the hygrothermograph placed in the heated bed show that the temperature was not kept high enough to inhibit the disease but in a very favorable range for its development and that the relative humidity under the cloth cover remained high for longer periods of time than in the unheated beds.

The four subsequent experiments were carried out in tightly constructed, well-insulated beds, subdivided by insulated walls into 3 x 6 foot sections. Various cover materials were used and several means of supplying heat were tried. The types of covers were: double glass sash; frames covered with the glass substitutes, Cel-O-Glass, Vio-Ray, and Flex-O-Glass; and heavy white cloth used without a frame. The following heat-producing units were placed directly in the beds: kerosene lanterns, electric light bulbs, and electric hot bed heating cables. In addition, a kerosene burner outside heated water which circulated through pipes within the bed.

Heat was supplied chiefly at night, except on a few cool rainy days. In the following experiments, thermostatically controlled heating cables or light bulbs were used as sources of heat. A maximum-minimum thermometer was used in each bed and in addition five thermographs were used in the eight beds. The minimum settings of the temperatures in beds at Florence were 34°C., 31°C., 29°C., and 21.5°C., respectively, and at Clemson 32°C., 28°C., 25°C., and 21°C., respectively. These were maintained at or above the minimum except on a few cold nights when the temperatures in some of the beds fell as much as 3°C. below the minimum for a short period in the early morning. The three high temperatures satisfactorily controlled the disease, although not completely. In the beds set at minimum temperatures of 34°C., and 32°C., the only symptoms of the disease that appeared were a few scattered non-fruiting spots which were restricted to very few plants. No evidence of damage to the plants could be noted in these beds. At a minimum temperature of 31°C., at Florence, about 40 per cent of the plants showed small non-fruiting spots on the leaves, which, however, did not appreciably affect the growth of the plants. At Clemson less than five per cent of the plants showed similar symptoms of the disease at this temperature. Minimum settings of temperatures of 29°C., 28°C., 25°C., and 21.5°C. were not sufficiently

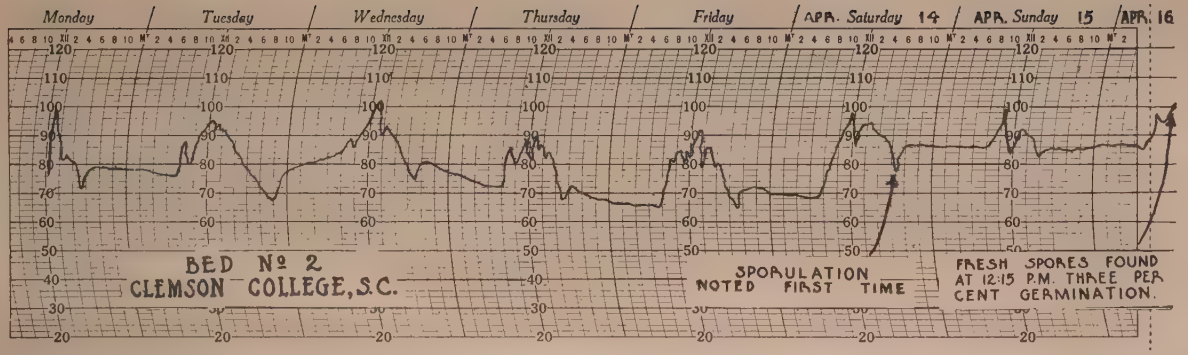

Figure 2.—A thermograph record from a plant bed in which fresh conidia were found April 16 though the temperature had not been below 83°F. (28°C.) since 7 P. M. April 14.

high to prevent sporulation of the fungus and spread of the disease. These temperatures delayed the appearance of the disease for approximately seven to 10 days, and considerably less damage occurred in these beds than in the unheated beds.

Clayton and Gaines (5) reported that a temperature above 21°C. would prevent sporulation and, therefore, further spread of the disease. In the controlled temperature of the temperature-humidity cases of our experiments, on the other hand, sporulation occurred at 25° to 26°C. Fresh spores, as shown by a germination test, were found in a plant bed on April 16 in which the temperature had not fallen below 83°F. (28°C.) since 7 P. M., April 14 (Figure 2.) A new thermograph from which the record was obtained was on the ground among the infected leaves. In a well insulated plant bed heated by a thermostatically controlled electric cable, there was enough fluctuation in temperature at a minimum setting of 29°C. to permit an appreciable damage from the disease. The disease was controlled satisfactorily, however, at minimum settings of 31°C. and above, but to maintain such high temperatures is expensive.

Glass sash retained heat better and allowed more normal plant growth than any of the covers. Plants grown under the heavy cloth were the least desirable since they were succulent and more severely injured by the fungus than hardened ones. Succulent plants are also undesirable for transplanting.

Kerosene lanterns were unsatisfactory in an enclosed bed, because of the formation of a heavy deposit of carbon and the untimely extinction of the flame as the oxygen in these beds was depleted.

No significant difference in plant growth or control of the disease could be noted between the cable-heated and the bulb-heated beds. The additional light from the electric light bulbs did not appreciably accelerate the growth of the plants.

An experiment with heated beds was conducted at Florence in cooperation with Mr. W. M. Lunn of the United States Department of Agriculture. Air heated by charcoal fires was conducted through flues, in one case a single flue placed underground in a bed 16 x 6 feet and in the other case two flues placed above ground along each side of a bed 25 x 6 feet. In both cases the end of the bed farthest from the heater was insulated from the rest of the bed and covered with two sections of standard double glass sash. The other parts of the beds were covered with heavy white cloth. The fires were started at about six o'clock in the afternoon and recharged again at midnight. A high temperature was maintained satisfactorily for about three to four hours after charging, but invariably dropped too low for disease control after that time. In these beds the disease appeared about 10 days later than in the nearby unheated beds. The damage to the plants, however, was slight, and the period required for recovery was not so long in these beds as in the others.

In general, it may be said that, although the disease can be satisfactorily controlled by maintaining a high temperature in the plant bed, all methods used for obtaining this temperature are too expensive for the average grower to use. It was necessary to heat most of the beds for about three weeks and the cost of heating was calculated on this basis. Figures on the cost of heating beds are approximate and, therefore, are not presented but it is believed that they are sufficiently accurate to justify the statement regarding the cost.

FORCED AIR VENTILATION

A small electric fan which forced outside air over a heating device and thence into the bed was used with the idea that it might reduce the relative humidity sufficiently to prevent infection and sporulation. The temperature of the bed seldom reached 20°C., so there was no complication of a temperature control of the disease. The first trial at Clemson successfully controlled the disease, probably because the plants were well developed with upstanding leaves. In the second trial at Clemson, the larger plants in the bed were protected but small plants with the leaves on the ground were killed in large numbers. A Friez hygrothermograph placed on the soil among the plants registered approximately 50 per cent relative humidity during the time the disease was most prevalent. Drops of water, however, could be seen on the lower side of small leaves on the ground which were four inches below the detecting hairs of the instrument that was registering only 50 per cent relative humidity. It was thus evident that the fan was not circulating the air around the small closely packed plants near the ground. The same method was tried at Florence concurrently with the second trial at Clemson. The warm air dried the sandy soil there to such an extent that the plants nearest the fan were badly wilted each morning and the experiment had to be discontinued after a week. These attempts at humidity control indicated the impracticability of preventing dew formation on small plants as they are massed in the average plant bed.

SPRAY MIXTURES

Control of the disease has been attempted with the following spray materials: 4-4-50 Bordeaux Mixture*, copper arsenite*, copper resinate*, copper phosphate*, Cal-Mo-Sul, copper oxide (red), and home-made colloidal copper (11). Careful observations of the spray experiments performed by Mr. Lunn in 1933, indicated that the substances he used gave unsatisfactory control of the disease. The re-

*Materials supplied by Dr. E. E. Clayton and used by Mr. W. M. Lunn at Florence in 1933. We were extended the privilege to observe the progress of the experiments.

port from Henderson (10) that Cal-Mo-Sul appeared to have possibilities in the control of the disease and a similar report from Mandelson (11) with reference to colloidal copper led to the use of these materials along with red copper oxide. During the spring of 1934, the sprays at Clemson with Cal-Mo-Sul at the rates of one and two ounces per gallon were encouraging. The disease more nearly approached the severity of the attack in 1932 in these tests than in any of the others. The plants were small when the disease was first noted and its presence was delayed for two weeks in the sprayed beds, though all beds finally appeared to be about equally infected. There were 12 sprayed and three unsprayed beds 3 x 6 feet in size. After recovery from the disease, a count of two representative unsprayed beds showed an average of 423 plants per bed as compared with 1465 in two representative beds sprayed with Cal-Mo-Sul at four-day intervals.

The above experiment corrobates the use of sprays as stressed in Australia, where the disease usually appears early enough to kill a very high percentage of plants. Under these conditions the use of sprays is advantageous. The sprays are used there, not to control the disease, but to delay its appearance long enough so that plants will attain a size sufficiently large to live through the inevitable attack. At Florence, however, in 1933 to 1935, inclusive, the disease did not spread until the plants were almost ready to be set in the field, few plants were killed, and the appearance of the disease was delayed on sprayed plants. As a result, the unsprayed plants had recovered sufficiently to be set in the field several days sooner than the sprayed plants.

During the fall of 1934, the spray test at Clemson included 10 beds sprayed with Cal-Mo-Sul, 10 with colloidal copper and 9 unsprayed. Each bed was 4 x 6 feet in size and the sprayed beds received six applications. Inoculations were made when the plants had almost reached a size suitable for transplanting. The disease appeared to be generally present throughout all the beds but after recovery, the average number of plants remaining in six representative beds from each treatment were as follows: unsprayed, 349; sprayed with Cal-Mo-Sul, 450; sprayed with colloidal copper, 554.

The degree of control of this disease at Clemson, while not approaching that obtained with some of the standard sprays for fruit diseases, was encouraging and a more extensive spray experiment was carried out at Florence during the spring of 1935. Cal-Mo-Sul, two ounces to the gallon of water, colloidal copper (11), and red copper oxide, ½ ounce per gallon with a Lethane spreader were used. Each spray was applied to 21 beds totalling 72 square yards, and 33 unsprayed beds totalling 105 square yards were frequently interspersed among the sprayed beds. Some of the beds were 3 x 6 feet, some 6 x 6 feet, and others 6 x 9 feet in size. Each spray was applied eight times at four-day intervals, using a bucket spray pump capable of developing 85 pounds pressure. The spray was applied from both sides of a bed, thus insuring as good a coverage

as is usually possible with the closely packed plants of a tobacco bed. The disease first appeared on April 11 in one corner of this area, and immediately the rest of the area was inoculated with these diseased leaves. The disease spread erratically, appearing in beds here and there apparently irrespective of treatment.

On April 25 and 26 the disease became very active and spread rapidly. On April 27, at the time the disease seemed to be most prevalent, the plants were counted in 25 one square-foot areas in each spray treatment and the controls. At the same time an estimate of the extent of infection was made on 20 plants pulled at random from each of the counted areas. The leaves of the plants thus selected were carefully examined and the total necrotic area of each plant was estimated. The plants were classified as follows: class 1, no symptoms of disease; class 2, necrotic areas averaging less than one-fourth of the total leaf area; class 3, approximately one-half the total leaf area; and class 4, total leaf area.

Table 2 gives the results of this estimate.

Table 2.—Extent of Disease on Sprayed and Unsprayed Plants Based Upon the Area of Necrotic Tissue per Plant

Type of area	Number of plants in each class			
	Class 1*	Class 2*	Class 3*	Class 4*
Unsprayed	0	139	335	26
Cal-Mo-Sul	19	263	206	12
Copper Oxide	4	237	249	10
Colloidal Copper	7	284	203	6

*1—No symptoms of disease
*2—Necrotic area averaging less than one-fourth total leaf area
*3—Necrotic area averaging approximately one-half total leaf area
*4—Total leaf area showing necrosis

It will be noted from Table 2 that practically every plant examined was infected to some extent. While the sprays did not prevent the disease they evidently reduced the number of infections since the number of sprayed plants in classes 3 and 4 was less than the number of unsprayed plants in these classes.

A count of plants made on April 27 showed approximately 40 plants per square foot in all beds. A final count on May 6, after the disease had apparently disappeared, showed insignificant variations from the first count, indicating that practically no plants had been killed.

GENERAL SUGGESTIONS FOR CONTROL

The following suggestions are made, as a result of experiences with the disease that seem to be of value in its control:

1. Select a warm sunny place for the bed which will not be shaded during any part of the day. The use of a new site or a thoroughly disinfected old site is a good precaution since over-wintering oospores in the old bed may initiate the disease.

2. Plant more bed space than is needed. Two or three beds in different locations are ordinarily better than one large bed.

3. Remove covers during the day to admit sunlight and air as soon as weather will permit.

4. Any practice that will hasten plant growth is helpful, since large plants are usually damaged less than small ones.

5. If desirable to increase the rate of growth of diseased plants, a moderate application of nitrate of soda may be beneficial.

6. Avoid dissemination of conidia by carrying them on hands or clothing from infested to non-infested beds.

7. If possible transplant to the field before the disease occurs in the bed. Do not transplant diseased plants until they have recovered.

SUMMARY

A short discussion of the history and symptoms of the disease is given. Observations on the morphology of the organism are also presented.

Pepper, tomato, and eggplant were infected by inoculation with conidia from tobacco. Tobacco has been infected by conidia taken from pepper.

Conidia were found to germinate at temperatures from 1° to 3°C. up to 29°C. Several instances of delayed germination were noted. Viable conidia were found in plant beds at all hours of the day. Numerous unsuccessful attempts have been made to germinate oospores.

Temperature-humidity cases used in some of the studies are described.

It is shown that spore germination, infection, and sporulation will occur at relative humidities slightly below the dew-point.

Infection has occurred at temperatures from 5° to 30°C. inclusive; 25° to 26°C. is the highest temperature at which the production of conidia occurred.

Plants which recovered from the disease have been reinfected in several instances though others similarly inoculated have failed to show symptoms of the disease.

During two winter seasons, plants protected in beds produced conidia periodically.

Experiments on the control of the disease by high temperatures, forced air ventilation, and spraying are presented. Minimum settings of the temperature at 31°C. in well insulated plant beds gave satisfactory control of the disease. Because of inevitable fluctuations, lower temperatures than 31°C. are likely to lead to appreciable damage from the pathogen.

Suggestions are made, as a result of experiences with the disease, that seem to be of value in its control.

LITERATURE CITED

1. Adam, D. B.
   BLUE MOULD OF TOBACCO. Jour. Dept. Agr. Victoria 31: 412-416. 1933. (Reprint pp. 1-5. 1933).
2. Angell, H. R. and Hill, A. V.
   DOWNY MILDEW (BLUE MOULD) OF TOBACCO IN AUSTRALIA. Counc. Sci. Ind. Res. Bull. 65. 1932.
3. Armstrong, G. M. and Albert, W. B.
   DOWNY MILDEW OF TOBACCO ON PEPPER, TOMATO, AND EGGPLANT. Phytopath. 23: 837-839. 1933.
4. Bressman E. N. and Nichols, R. A.
   GERMINATION OF THE OOSPORES OF PSEUDOPERONOSPORA HUMULI. Phytopath. 23: 485-487. 1933.
5. Clayton, E. E. and Gaines, J. G.
   CONTROL OF DOWNY MILDEW DISEASE OF TOBACCO THROUGH TEMPERATURE REGULATION. Science. N. S. 78: 609-610. 1933.
6. Clayton, E. E. and Stevenson, John A.
   NOMENCLATURE OF THE TOBACCO DOWNY MILDEW FUNGUS. Phytopath. 25: 516-521. 1935.
7. Darnell-Smith, G. P.
   INFECTION EXPERIMENTS WITH SPORES OF BLUE MOULD DISEASE OF TOBACCO. Agr. Gaz. N. S. W., 40: 407-408. 1929.
8. Dixon, L. F., McLean, Ruth A., and Wolf, F. A.
   THE INITIATION OF DOWNY MILDEW OF TOBACCO IN NORTH CAROLINA IN 1934. Phytopath. 25: 628-639. 1935.
9. Farlow, W. G.
   NOTES ON SOME INJURIOUS FUNGI OF CALIFORNIA. Bot. Gaz. 10: 346-348. 1885.
10. Henderson, R. G.
    EXPERIMENTS ON CONTROL OF DOWNY MILDEW OF TOBACCO. (Abst.) Phytopath. 24: 11. 1934.
11. Mandelson, L. F.
    ADDITIONAL RECOMMENDATIONS FOR THE CONTROL OF BLUE MOULD OF TOBACCO. Queensland Agr. Jour. 40: 465-469. 1933.
12. Miller, L. P.
    EFFECT OF SULPHUR COMPOUNDS IN BREAKING THE DORMANCY OF POTATO TUBERS AND IN INDUCING CHANGES IN THE ENZYME ACTIVITIES OF THE TREATED TUBERS. Contrib. Boyce Thompson Inst. 5: 29-81. 1933.
13. Smith, E. F. and McKenney, R. E. B.
    A DANGEROUS TOBACCO DISEASE APPEARS IN THE UNITED STATES. U. S. Dept. Agr. Circ. 174. 1921.
14. Spegazzini, Carlos.
    PHYCOMYCETEAE ARGENTINAE. Rev. Argentina Hist. Nat. 1: 28-38. 1891. (Cited by Wolf et al.)
15. Stevens, N. E.
    A METHOD FOR STUDYING THE HUMIDITY RELATIONS OF FUNGI IN CULTURE. Phytopath. 6: 428-432. 1916.
16. Stoughton, R. H.
    APPARATUS FOR THE GROWING OF PLANTS IN A CONTROLLED ENVIRONMENT. Ann. Appl. Biol. 17: 90-106. 1930.
17. Tryon, Henry.
    TOBACCO DISEASES. Leaflet. Queensland Dept. Agr. June 19, 1890. (Cited by Angell and Hill.)
18. Wolf, F. A., Dixon, L. F., McLean, Ruth, and Darkis, F. R.
    DOWNY MILDEW OF TOBACCO. Phytopath. 24: 337-363. 1934.

Bull. S.C. agric. Exp. Sta.